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by

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PCN 351SQ

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ABSTRACT

A sandwich immunoassay for Newcastle Disease Virus (NDV) was developed using streptavidin-biotin mediated filtration capture and silicon-based semiconductor detection. The assays were carried out in one step whereby the immunoreagents and the antigen (NDV) were mixed together and incubated. The pH sensing capability of the detector, a light addressable potentiometric (LAP) sensor, was employed to detect the presence of immobilized urease-conjugated antibodies. Lower limits of detection (LOD) of the assay were determined as a function of incubation time. The LOD were approximately 27, 15, and 3 ng per well for 1, 5, and 60 minute incubations, respectively. The techniques described here have the potential to be adapted to an automated LAP sensor for use in the BioChemical Detector program.

RÉSUMÉ

Un test immunologique de type «sandwich» pour la détection du virus de la maladie de Newcastle (NDV) a été mis au point. Le test utilise la capture par filtration médiée par le système streptavidine-biotine et la détection par semi-conducteurs au silicium. Le mélange des immunoréactifs et de l'antigène (NDV) ainsi que leur incubation s'est fait en une seule étape. La sensibilité au pH du détecteur, un titrimètre adressable par la lumière (TAL), a permis de déceler la présence d'anticorps immobilisés conjugués à l'uréase. Les limites inférieures de détection (LID) ont été déterminées en fonction du temps d'incubation. Les LID on été approximativement de 27, 15, et 3 ng par puits pour des durées d'incubation de 1, 5 et 60 min respectivement. Les techniques qui ont été employées peuvent probablement être adaptées à un TAL automatique pour usage dans le Programme de détecteurs biochimiques (BioChemical Detector program).

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INTRODUCTION

At the present time there is no device for the rapid detection of biological agents in the field. The BioChemical Detector (BCD) program is a collaborative research and development effort among the United States, the United Kingdom and Canada and has as its goal the development of an automated portable detector capable of detecting a range of chemical and biological threat agents. The physical specifications of the BCD were set to 1 cu. ft (ca 0.03 m^3) in volume, 10 lb (ca 5 kg) in weight and 2-5 minute assay time for collection, treatment of sample, analysis and results.

The biosensor module of the BCD is to have the capability of detecting proteins, viruses and bacteria. It is an immunochemically-based instrument which performs the analyses by an enzyme-linked immunosorbent assay (ELISA) (1). The biosensor for the BCD was designed under contract with the Chemical Research, Development and Engineering Center of the U.S. Army (Aberdeen, MD) by Environmental Technologies Group (Baltimore, MD). At the BCD design review meeting in June 1989 it was decided that the technology for the BCD biological detection module was to be a light-addressable potentiometric (LAP) sensor (2, 3). This is a new technology produced by Molecular Devices Corporation (Menlo Park, CA). The LAP sensor was available to the BCD program in two forms, a custom made module for the BCD breadboard, and a commercially available unit, marketed under the trade name of Threshold Unit™. The latter had a wider range of analytical capabilities, was more "user friendly" and in general was better equipped for the development of LAP sensor assays. The breadboard model had a limited number of features, i.e., those specifically required for the automatic operation of the BCD.

The work presented in this report was carried out exclusively on the Threshold Unit. Since the technology was the same in each device, the lessons learned on the Threshold Unit could be directly applied to the BCD breadboard sensor.

Sensors based on silicon fabrication technology provide a number of advantages over conventional detectors for biochemical analysis. These include high sensitivity, the capacity for miniaturization and the availability of multiple measurement sites (4). When coupled with enzyme immunoassay technology, silicon semiconductor sensors are a powerful analytical tool.

Among the international commitments that Canada has to the BCD program is the development of an assay for virus on the LAP sensor. The virus of choice for the initial work was the La Sota vaccine strain of Newcastle Disease Virus (NDV). It is a viable agent and has been approved by Agriculture Canada for dissemination in the open air. This virus was selected by DRES for use as a BW simulant (5) and it will be used for future field trials of the BCD at DRES.

The objective of this work was to develop one-step immunoassays for NDV on the Threshold Unit, assays which could be transposed to the BCD breadboard. Of primary concern was the relationship between the lower limits of detection and the assay time.

MATERIALS and METHODS

Reagents

Bovine serum albumin (BSA), sodium chloride, sodium dihydrogen phosphate, Tween 20, Triton X-100 and urea were obtained from Sigma Chemical Co. (St Louis, MO) and used without further purification. Streptavidin was obtained from Scripps Laboratories (San Diego, CA). It was reconstituted in distilled water to yield a stock concentration of 10 mg/ml. Hybridoma clones producing anti-NDV monoclonal antibodies were produced under DND contract by the University of Alberta (Edmonton, AB). Antibody from clone 25R5 was covalently linked to urease; antibody from clone 55R3 was covalently linked to biotin. The conjugations of the antibodies with urease and biotin were carried out under contract by J. D. Biologicals (Scarborough, ON) and the conjugated antibodies were used without further purification. The protein concentrations of the urease and biotin conjugated antibodies, as determined by the contractor, were 0.50 and 0.46 mg/ml, respectively. The antigen, La Sota strain of NDV was grown, in-house, in allantoic fluids, purified by sucrose gradient centrifugation (6) and suspended in phosphate buffered saline pH 7.0. The protein concentation of the NDV stock solution was 1 mg/ml, determined spectrophotometrically with a BCA protein assay kit (Pierce Chemical Co., Rockford, IL).

Wash solution consisted of 150 mM NaCl, 10 mM phosphate buffer, pH 6.5, plus 0.2% Tween 20 detergent. The dilution buffer was the wash solution titrated to pH 7.0, containing 1% albumin and 0.25% Triton X-100. The substrate solution for the enzyme assays was wash solution containing 100 mM urea.

Apparatus

The apparatus for these assays was a commerically available LAP sensor, marketed under the name Threshold Unit™. It was purchased from the manufacturer, Molecular Devices Corp. (Menlo Park, CA). The instrument was controlled by an IBM PS/2 model 30 microcomputer and custom designed software supplied by Molecular Devices Corp. The design of the Threshold Unit allowed eight samples to be tested simultaneously. Nitrocellulose membrane filters (0.44 μm pore size) coated with biotinylated BSA were purchased from Molecular Devices Corp.

Immunoassay Procedures

A reagent solution consisted of 50 μl urease conjugated anti-NDV, 100 μl biotinylated anti-NDV, 40 μl streptavidin and 12 ml dilution buffer. The reagent solution was allowed to stand at room temperature for 3 hr prior to use. A set of calibration standards of NDV was prepared by diluting the stock solution of NDV with dilution buffer to provide standards ranging typically 1.5 - 100 ng per well.

Figure 1 provides a schematic representation of the sandwich immunoassay for NDV. A volume of 150 μl of reagent solution was added to each 100 μl aliquot of NDV sample. The reagents and virus were mixed thoroughly and incubated at room temperature for the required length of time. At the end of the incubation period, a portion of the incubated sample-reagent mixture, 150 μl, was delivered to a well of the filter assembly of the Threshold Unit. This aliquot of 150 μl contained the equivalent of 60 μl of NDV sample and 90 μl of reagent solution.

Filtration Capture and Potentiometric Sensing

The sample-reagent mixture was filtered through the biotinylated nitrocellulose membrane at 250 μl per min. Biotin-streptavidin interactions (7) mediated the capture of the sandwich antibody-antigen complexes on the membrane. The streptavidin in the reagent solution acted as the binding agent to immobilize the antigen-biotinylated antibody complexes on the membrane. The membrane was then washed with 0.5 ml of wash solution and the filtration rate was increased to 750 μl per min.

The membrane stick containing immobilized antigen-antibody complexes was removed from the filter assembly and inserted into the reader compartment of the Threshold Unit which contained the LAP sensor and the urea substrate solution. A plunger pressed the membrane against the surface of the silicon sensor. The instrument was designed so that the spots on the surface of the membrane which contained immobilized antigen-antibody complexes aligned with the pH sensitive measurement sites on the surface of LAP sensor. At the surface of the sensor the hydrolyis of urea to carbon dioxide and ammonia caused an increase in the pH which was detected as a change in the surface electropotential. The data were recorded and stored on the microcomputer using the custom designed software. The rate of change of pH at the surface of the silicon sensor was monitored by the rate of change with respect to time of the surface potential as μv/sec.

RESULTS AND DISCUSSION

Signal-to-Noise Ratio

In this work, the sandwich antibody-antigen complexes that constituted the products of the immunoreactions were derived from one-step incubations. In the reaction scheme of the assay (Figure 1), the rate of change of pH vs time on the membrane and hence the output of the LAP sensor, will depend upon the number of urease-containing antibody-antigen sandwiches immobilized during the filtration capture process. Since the efficiency of the biotin-mediated filtration capture of streptavidin is high, approximately 95% under the conditions of these assays (8), the number of the urease-containing antibody-antigen sandwiches immobilized will be a measure of the number formed during the incubation period.

A series of immunoassay experiments was undertaken to investigate the effect of the incubation time upon the output response of the LAP sensor. The aspect of the response of interest was the signal-to-noise ratio (S/N) and its variation with respect to the length of the incubation time of the antibodies and antigens. The noise component of the ratio (N) was the background of the assay, that is, the output of the LAP sensor for the reagents alone, with no antigen. The signal component (S) was the output of the LAP sensor from a sample of 50 ng NDV per well, less the noise component. For a given incubation time, four of the test sites of the capture membrane contained the NDV samples, the other four sites were reserved for the reagents alone (noise).

The test samples were incubated at room temperature for periods of time ranging from one minute to two hours and the values of S/N of the assays were plotted as a function of incubation time (Figure 2). There was a continuous increase in S/N up to and in-

cluding the longest incubation time (125 min). The value of S/N at 125 min was 6.6 which appears close to a maximum. Extrapolation of the data to times greater than 125 min would result in S/N values that are comparable to 6.6.

A comparison of S/N of the 1 and 125 min assays shows that the one minute assay of this format generates only about 8% (0.5/6.6) of the total potential signal. A comparison of the data of the 5 and 125 min assays shows that the fraction of the potential signal rises to about 17% (1.1/6.6).

The reactions involved in immunoassays are not "instantaneous". Typical values of the association constant, k_a, for antibody-antigen interactions are 10^8 M^{-1} sec^{-1} (9, 10). On inspection, these values may seem large but when the working concentrations of the antibodies are taken into consideration, for example, 10^{-10} - 10^{-11} M, the overall reaction time for the system to approach equilibrium (90% of maximum value) is in the order of an hour or greater. In order to obtain maximum sensitivity in immunoassays, incubation steps are generally about one hour (11).

The variation of S/N with incubation time is an important consideration for the future development of the BCD program. The goal of the program is to produce a rapid detection system for biological agents using antibody immunoassay technology. In the design of the BCD, the time allotted to incubation of the antibodies and antigens is about one to two minutes. From the data shown here it can be seen that short incubations severely limit the amount of signal that can be derived from an assay.

The Response of the LAP Sensor to NDV

The response of the LAP sensor was monitored over a large range of NDV concentrations per well. One-step immunoassays are generally susceptible to a "hook effect" at high amounts of antigen, whereby increasing amounts of antigen produce decreasing signals (12). This occurs when the antibody reagents are no longer in excess and the increased amounts of antigen diminish the probability of the formation of antibody-antigen sandwiches. The results of a representative assay with NDV concentrations ranging from 38 ng to 10 μg are shown in Figure 3. The output of the LAP sensor (μv/sec) increased monotonically as a function of antigen concentration; there was no "hook effect" observed in the assay although the slope of the curve (i.e., sensitivity of the assay) was reduced at the higher antigen concentrations. These results suggested that the concentration of reagents selected for use in this and subsequent NDV assays was adequate to accommodate, without becoming overloaded, antigen concentrations up to 10 μg per well. False negative responses of the LAP sensor due to large antigen excesses were considered unlikely.

Limits of Detection of the LAP Sensor for NDV

The values of the lower limits of detection (LOD) of the LAP sensor for NDV were determined at three incubation times: 1, 5 and 60 min. The results of a one-minute assay of NDV are presented in Figure 4. The standards on the calibration curve ranged from 5 ng to 200 ng per well and were run simultaneously on a single capture membrane stick. Each data point represents the mean of three consecutive assays performed on the same day using the same reagents. The data, μv/sec versus ng of antigen, was represented well by a linear plot. The error associated with the individual points was small: the coefficient of

variation (cv), defined as the ratio of the standard deviation (SD) to the mean was about 5%. The LOD of the assay was 20 ng per well. The LOD was determined from the sum of the mean background (zero antigen) plus twice the standard deviation on the background and was taken to be the intersection of the calibration curve with background+2SD. Two subsequent assays of NDV with one-minute incubations were performed using freshly prepared antibody and antigen solutions. Employing the criteria described above, the LOD of these assays (data not shown) were 30 and 35 ng per well. The mean LOD (three determinations) for the one-minute assay therefore was approximately 27 ng per well.

A five-minute assay of NDV was carried out in a similar manner (Figure 5) and the LOD determined from the data was 15 ng per well. Two subsequent determinations of the LOD employing freshly prepared antigen and antibody solutions (data not shown) gave estimates of 18 and 12 ng per well. The mean LOD for the three assays was 15 ng per well.

For the sixty-minute assay of NDV, the LOD was 3 ng per well (Figure 6). Two additional sixty-minute assays with freshly prepared antigen and antibody solutions (data not shown) gave LOD of 4 and 2 ng per well and a mean (three determinations) of 3 ng per well. The results of the sixty minute assay compare favourably with a multistep sandwich chromogenic ELISA for NDV having incubation times of one hour per step in which the LOD was about 1 - 2 ng per well (11).

In the present work the detectability (the ability to detect lesser amounts of analyte) is defined as the reciprocal of LOD and is used here in a comparative context. That is, if the LOD of assay A is one-half that of assay B, then the detectability of A is twice that of B. In this work the detectability of the 5 min and 60 min assays was about 2 and 10 fold greater than that of the 1 min assay. There was a good correlation (r^2 = 0.99) between detectability and S/N (Figure 7).

Variations in slopes and intercepts of the calibration curves were observed, indicating that the overall output of the LAP sensor displayed some day-to-day fluctuations. The output (μv/sec) depends upon the rate of hydrolyis of urea by the enzyme, urease, immobilized at the surface of the nitrocellulose membrane. Rates of hydrolysis are influenced by such factors as ambient temperature and pH of the buffers. Small day-to-day differences in these factors as well as the use of different stocks of conjugated antibodies on different days may have combined to account for the observed variations. The day-to-day variation of the slope for a given incubation time was about 20% while that of the background was somewhat higher, about 50% (data not shown). Despite the variations in slopes and intercepts from day-to-day, the LOD were reasonably constant.

Detectability of NDV by Precision Sticks

Precision stick was the term used to denote a membrane stick having replicate samples in the test sites. For a particular precision stick, four of the eight wells received fixed amounts of NDV and antibody solutions, the other four wells received the antibody solution only and served as the background or reference. The means and standard deviations of the output of the LAP sensor for the NDV-containing samples and for the backgrounds were calculated. For a given stick, the output signal was considered positive if the mean of the sample containing NDV was greater than the mean output plus twice the standard deviation of the background.

For one-minute incubations, precision sticks were tested for 80, 40, 20 and 10 ng per well. The concentrations were chosen with reference to the LOD given by the calibration curves (e.g., Figure 4). A value of 80 ng per well was within the detectable range of a one-minute assay, 10 ng per well was out of this range and 40 and 20 ng per well were of the order of the LOD. The data (Table I) shows that the samples of 40 ng NDV per well were the

lowest concentration which produced sensor outputs greater than the respective backgrounds + 2SD. Thus the detectable limit of NDV was between 20 - 40 ng per well. These results are consistent with the mean LOD obtained from the calibration curves of the one-minute assay, viz., 27 ng. For five-minute and sixty-minute incubations, precision sticks were assayed for ranges of NDV concentrations that bracketed the LOD estimated from the calibration curves (Tables II and III). The lowest concentrations of NDV giving sensor outputs greater than background + 2 SD were 10 and 2 ng per well for incubation times of five and sixty minutes, respectively. Overall there was good agreement between the results obtained from the calibation curves and from the precision sticks.

Although the BCD will use a LAP sensor detection system, assays will not be carried out using calibration curves. Rather, the output of a test site on the sensor will be compared to the output of a reference site in order to detect the presence of analyte, in a manner that is similar to that used with the precision sticks. These experiments suggest that, in principle, the LOD of an immunoassay determined on a commercial LAP sensor from calibration curves will be essentially the same as that obtained from precision sticks.

The ratio of sensor output of the sample to the background (s/b) for each of the lowest detectable concentrations of NDV in the precision stick assay, that is, 40, 10 and 2 ng per well for 1, 5 and 60 min incubations, was aproximately equal to 1.10 (Tables I, II and III). For the calibrations curves the ratios of the sensor output at the LOD to the background were 1.11, 1.22 and 1.21 for 1, 5 and 60 min incubations, respectively. The values of s/b at the LOD for these and other calibration-curve data (not shown) were nearly constant, in the range 1.1 - 1.2, even though the incubation times varied from one to sixty minutes. These results suggest that the method of detection of the BCD, comparing the output of a test site to that of a reference, is feasible. The data presented in this work were collected over a period of five months and there was no large amount of variation in s/b at the LOD for the assays.

Quantitation of NDV on the LAP Sensor

The ability of the LAP sensor to provide quantitative analysis of NDV was demonstrated. Series of calibration standards and test samples were prepared and then assayed on the LAP sensor. There was good agreement between the measured values of the test samples and the actual concentrations. For eight test samples of NDV in the range of 2 - 40 ng per well, the mean difference between the measured and the actual amounts was about 9%. A linear regression analysis yielded a high correlation (r^2 = 0.999) between the measured and actual amounts (Figure 8).

Correction for the Instrumental Sensitivity Bias of the LAP Sensor

The output of the LAP sensor had a noticeable instrumental bias. The output of the sensor from sample wells #1, 4 and 6 was generally higher than the mean while wells #2, 5, 7 and 8 produced outputs that were lower than the mean. The origin of the bias was believed to be in the silicon chip of the nitrocellulose membrane reader, rather than in the liquid handling and filtration capture procedures (13). In an attempt to correct for this bias, a series of ten precision sticks was assayed. For a particular stick, the amounts of analyte (for these experiments, NDV) and reagents were the same in each of the sample wells. On a given stick, the output for each test site was normalized to the mean output for that stick and the standard deviation and percent coefficent of variation were calculated for each stick. The same determinations, i.e., mean, SD and %cv were made for each test site in the series, that is, the mean of the ten values for site #1, mean for site #2, etc. (Table IV).

An analysis of variance between the individual rows in Table IV yielded a confidence level in excess of 99.9% that the variations (from 1.00) of the means of the rows were not the result of random fluctuations. To alleviate the instrumental bias, the mean outputs (i.e., the values in the "mean" column of Table IV) were used as correction factors. The output for a test site of a membrane stick was divided by the respective correction factor. When these correction factors were applied to the precision stick data in Table IV, the mean %cv (per stick) decreased to 5.3% from 9.3%. When these correction factors were used with other independent precision stick data (not shown), the variations always decreased. For calibration curves such as those shown in Figures 4-6, application of correction factors always increased the correlation coefficients of the linear plots.

The monoclonal antibodies selected for this study were directed against different epitopes on the virus envelope, as determined by a competitive fluorogenic ELISA (14). Therefore there was no competition between the urease and biotin conjugated antibodies for attachment sites on the surface of NDV. The LAP sensor assays typically contained 0.2, 0.3, and 3 μg per well of the antibody-urease conjugate, antibody-biotin conjugate and streptavidin, respectively. These amounts provided an excess of the conjugated antibodies over the amount of NDV present in the samples used in the calibration-curves and precision-stick assays, as well as a molar excess of streptavidin over the antibody-biotin conjugate. Empirically it was found (data not shown) that a molar excess of the antibody-biotin conjugate over the antibody-urease conjugate produced favourable results. The concentrations of the reagents were chosen to provide backgrounds in the range of 75 - 250 μv/sec. These tended to give the most reproducible results with the least amount of scattering of

the data for assays having a large range of NDV concentrations. In this work, a single formulation of the reagent solution was successfully used on NDV samples ranging from 2 ng to 10 μg per well and for incubation times from one minute to two hours.

The biotin-streptavidin mediated filtration onto nitrocellulose provided an efficient separation of antibody-antigen complexes from the liquid phase. When coupled with one-step incubations, short assay times are readily obtained. Immobilization onto biotinylated nitrocellulose can be used with a variety of assays where an analyte-binding molecule can be labelled with one of the biotin-streptavidin components (15). Biotin-streptavidin immobilization onto nitrocellulose in conjuction with LAP sensor detection has been successfully employed in assays for proteins (16, 8) and for DNA (17).

CONCLUSIONS

We have described a rapid one-step sandwich format immunoassay for NDV. This work represents the first detailed report of the use of LAP sensor technology for the detection of a virus. The antigen could be detected with confidence over a large range, from 2 ng to 10 μg per sample. At the concentrations of antigen and reagents used, there was no "hook effect", i.e., there were no falsely low determinations at high concentrations antigen. The assays were carried out with one-, five-, and sixty-minute incubations and in general, the longer incubations produced lower LOD. Over the course of the work (about six months), the LOD of the assays were constant, suggesting that there had been no deterioration of the reagents during storage.

This work has demonstrated that, with respect to the BCD, LAP sensor technology is an adequate detection system for virus. The assay format described requires relatively few experimental steps on the part of the operator: mixing of the antigen and antibodies,

filtration capture, a single wash of the membrane, and detection of pH change on the membrane and, in principle, is transposable to the automated breadboard model of the BCD. The methodology used herein will be applied to future work in developing LAP sensor assays for other antigens of interest to the BCD.

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FIGURE CAPTIONS

Figure 1. The schemes shown provide a representation of the reactions occurring in the one-step immunoassays used in the LAP sensor.

Figure 2. The signal-to-noise ratio (S/N) vs incubation time for the LAP sensor assay of NDV. The noise component of the ratio (N) was the background (i.e., no antigen), the signal component (S) was the output for a 50 ng sample of NDV less the noise. Each point represents the quotient of the mean signal (n = 4) and the mean noise (n = 4). The error bars represent ±sum of 1 SD of the signal and 1 SD of the noise.

Figure 3. The response of the LAP sensor to NDV. Each point represents the mean of three determinations. The error bars, where not masked by the data-point markers, represent ± 1 SD.

Figure 4. Assay of NDV employing a 1-minute incubation. Each point represents the mean of three determinations and the error bars represent ± 1 SD. The line defining the calibration curve is given by 76.3 + 0.54x, r^2 (correlation coefficient) = 0.989. The LOD (20 ng) was taken to be the intersection of the calibration curve with the background plus 2 SD.

Figure 5. Assay of NDV employing a 5-minute incubation. Each point represents the mean of three determinations and the error bars represent ± 1 SD. The line defining the calibration curve is given by 262 + 4.2x, r^2 = 0.987. The LOD (15 ng) was taken to be the intersection of the calibration curve with the background plus 2 SD.

Figure 6. Assay of NDV employing a 60-minute incubation. Each point represents the mean of three determinations and the error bars represent ±1 SD. The line defining the calibration curve is given by 68.8 + 7.52x, r^2 = 0.998. The LOD (3 ng) was taken to be the intersection of the calibration curve with the background plus 2 SD.

Figure 7. A plot of detectablity versus S/N for LAP sensor response to NDV. The values of detectablity (reciprocal of LOD, see text) and S/N (from Figure 1) are relative to those of the 1 min assay.

Figure 8. Quantitation of NDV on the LAP sensor. Each point represents the mean (n = 4) of an independent determination of NDV concentration. The error bars represent ±1 SD.

Table I

Precision Stick Data for One Minute Assay of NDV

NDV ng/well	sample (μv/sec)			background (μv/sec)			+2SD[b] (μv/sec)	s/b[c]	mean s/b
	mean	SD	%cv[a]	mean	SD	%cv			
80	149.2	10.0	6.7	123.0	6.7	5.5	136.5	1.21	122
80	125.6	1.6	1.3	103.3	7.2	6.9	117.6	1.22	
40[d]	123.4	9.2	7.4	112.4	5.5	4.9	122.8	1.10	1.10
40	131.7	9.5	7.2	120.5	5.3	4.4	131.1	1.09	
20	130.6	7.0	5.4	113.8	10.8	9.5	135.3	1.15	1.10
20	142.9	6.4	4.5	132.4	7.4	5.6	147.6	1.07	
10	121.4	7.3	6.0	115.5	11.4	9.9	138.4	1.05	1.07
10	101.4	9.3	9.2	92.4	7.3	7.9	107.1	1.09	

[a]The values of the LAP sensor output for each sample and background are the mean (n = 4), SD and percent coefficient of variation (SD/mean).

[b]+2SD represents the background plus 2×SD.

[c]s/b is the ratio of the output of the sample to background.

[d]The lowest detectable concentration was taken to be the lowest ng sample to produce an output greater than the background + 2SD. The lowest detectable concentration of NDV for a one minute assay was between 20 and 40 ng per well.

Table II

Precision Stick Data for Five Minute Assay of NDV[a]

NDV ng/well	sample (μv/sec)			background (μv/sec)			+2SD (μv/sec)	s/b	mean s/b
	mean	SD	%cv	mean	SD	%cv			
40	134.9	9.1	6.7	93.3	4.5	4.8	102.3	1.45	1.36
40	155.6	10.2	6.6	122.2	4.7	3.8	131.5	1.27	
20	123.7	8.2	6.7	103.0	2.3	2.2	107.5	1.20	1.16
20	129.6	5.8	4.5	115.8	3.8	2.7	122.2	1.12	
10[b]	140.7	4.3	3.1	129.8	1.0	0.8	131.8	1.08	1.09
10	142.7	3.7	2.6	130.8	1.3	1.0	133.3	1.09	
5	114.8	5.6	4.9	107.2	4.1	3.8	115.4	1.07	1.04
5	127.1	4.8	3.8	126.4	2.8	2.2	132.1	1.01	

[a]see footnotes of Table I.

[b]The lowest detectable concentration of NDV for a five minute assay was about 10 ng per well.

Table III

Precision Stick Data for Sixty Minute Assay of NDV[a]

NDV ng/well	sample (μv/sec)			background (μv/sec)			+2SD (μv/sec)	s/b	mean s/b
	mean	SD	%cv	mean	SD	%cv			
10	161.9	6.6	4.1	102.8	3.8	3.7	110.1	1.57	1.57
10	207.3	7.7	3.7	131.8	4.3	3.2	140.3	1.57	
4	162.1	4.8	3.0	127.9	5.0	3.9	137.8	1.27	1.20
4	132.1	2.8	2.1	116.4	5.1	4.4	126.7	1.13	
2[b]	168.6	6.0	3.6	156.2	7.3	4.7	168.2	1.08	1.11
2	139.1	5.5	3.9	122.7	6.6	5.4	135.9	1.13	
1	95.7	5.6	5.8	93.8	8.25	8.8	110.3	1.02	1.04
1	104.3	5.7	5.5	97.9	6.0	6.1	109.9	1.06	

[a]see footnotes of Table I.

[b]The lowest detectable concentration of NDV for a sixty minute assay was about 2 ng per well.

Table IV

Correction Factors for the Instrumental Sensitivity Bias of the LAP Sensor

Site #	Precision Stick # #1	#2	#3	#4	#5	#6	#7	#8	#9	#10	mean* (per position)	SD	%cv
1	1.10	1.11	1.13	1.13	1.00	1.20	1.02	1.14	1.08	1.09	1.10	0.06	5.3
2	0.85	0.89	0.88	0.86	0.88	0.99	0.93	0.91	0.85	0.93	0.89	0.04	4.9
3	0.98	0.95	1.02	1.02	0.98	1.04	0.98	0.95	1.03	1.02	1.00	0.03	3.3
4	1.03	1.10	1.04	1.05	1.00	1.01	1.04	1.08	1.03	0.98	1.04	0.04	3.5
5	0.98	0.88	0.87	0.88	1.01	1.06	0.99	0.93	0.90	0.94	0.94	0.06	6.8
6	1.12	1.14	1.01	1.10	1.13	1.08	1.15	1.04	1.21	1.09	1.11	0.06	5.2
7	0.92	0.93	1.06	1.04	0.93	0.86	0.98	0.92	0.91	0.94	0.95	0.06	6.5
8	1.02	1.00	0.97	0.91	1.08	0.82	0.92	1.02	0.99	1.05	0.98	0.08	7.7
mean[a]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
											mean %cv (per position) = 5.4		
SD	0.09	0.10	0.09	0.10	0.08	0.12	0.07	0.08	0.12	.072			
%cv	9.0	10.5	8.8	10.2	7.7	12.4	7.2	8.5	11.7	7.2			
mean %cv (per stick) = 9.3													

*the values in this column were used as correction factors to alleviate sensitivity bias of the membrane reader of the LAP sensor.

[a]The output for each precision stick was normalized to the mean for that stick.

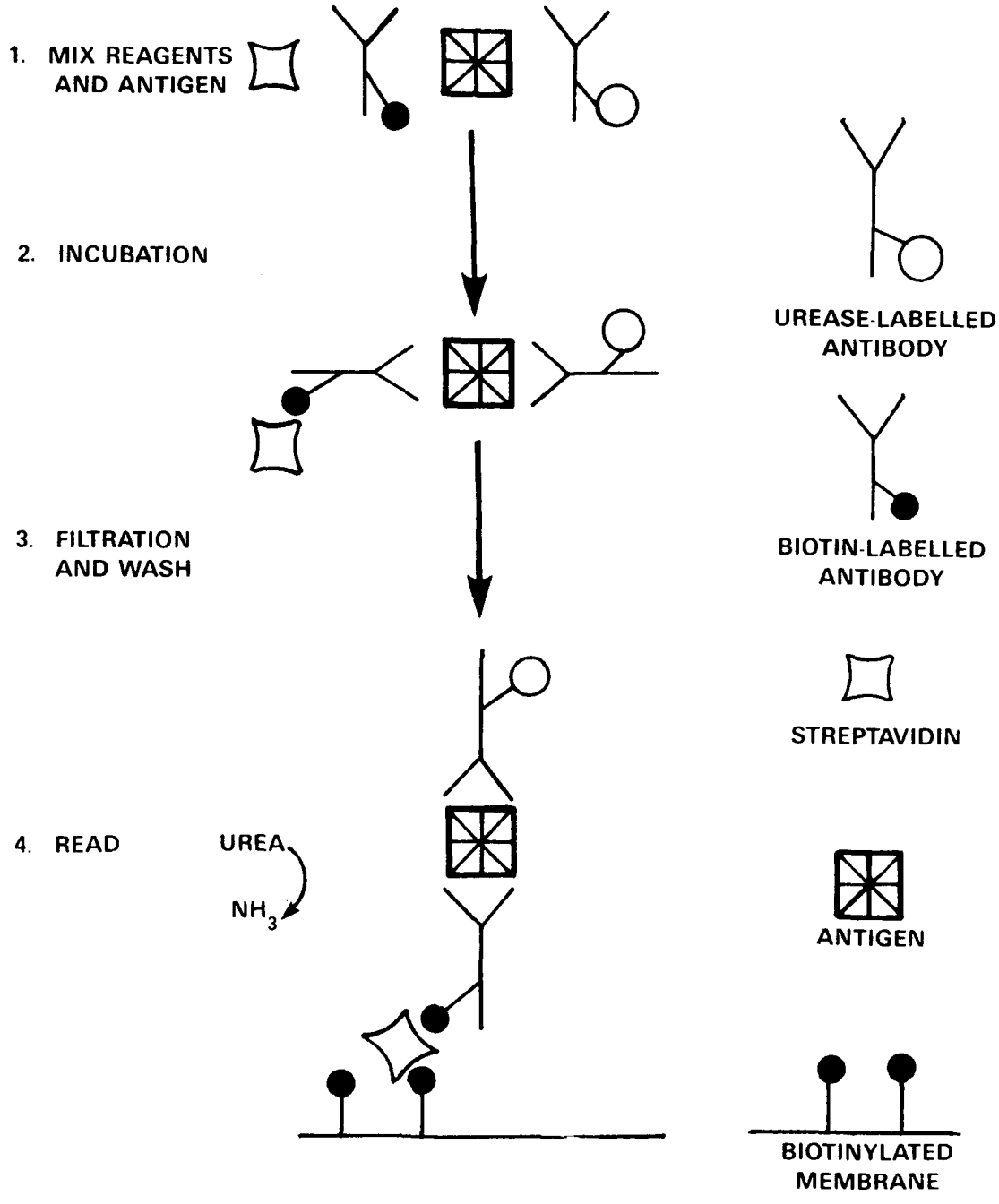

Figure 1

THE SCHEMES SHOWN PROVIDE A REPRESENTATION OF THE REACTIONS OCCURRING IN THE ONE-STEP IMMUNOASSAYS USED IN THE LAP SENSOR.

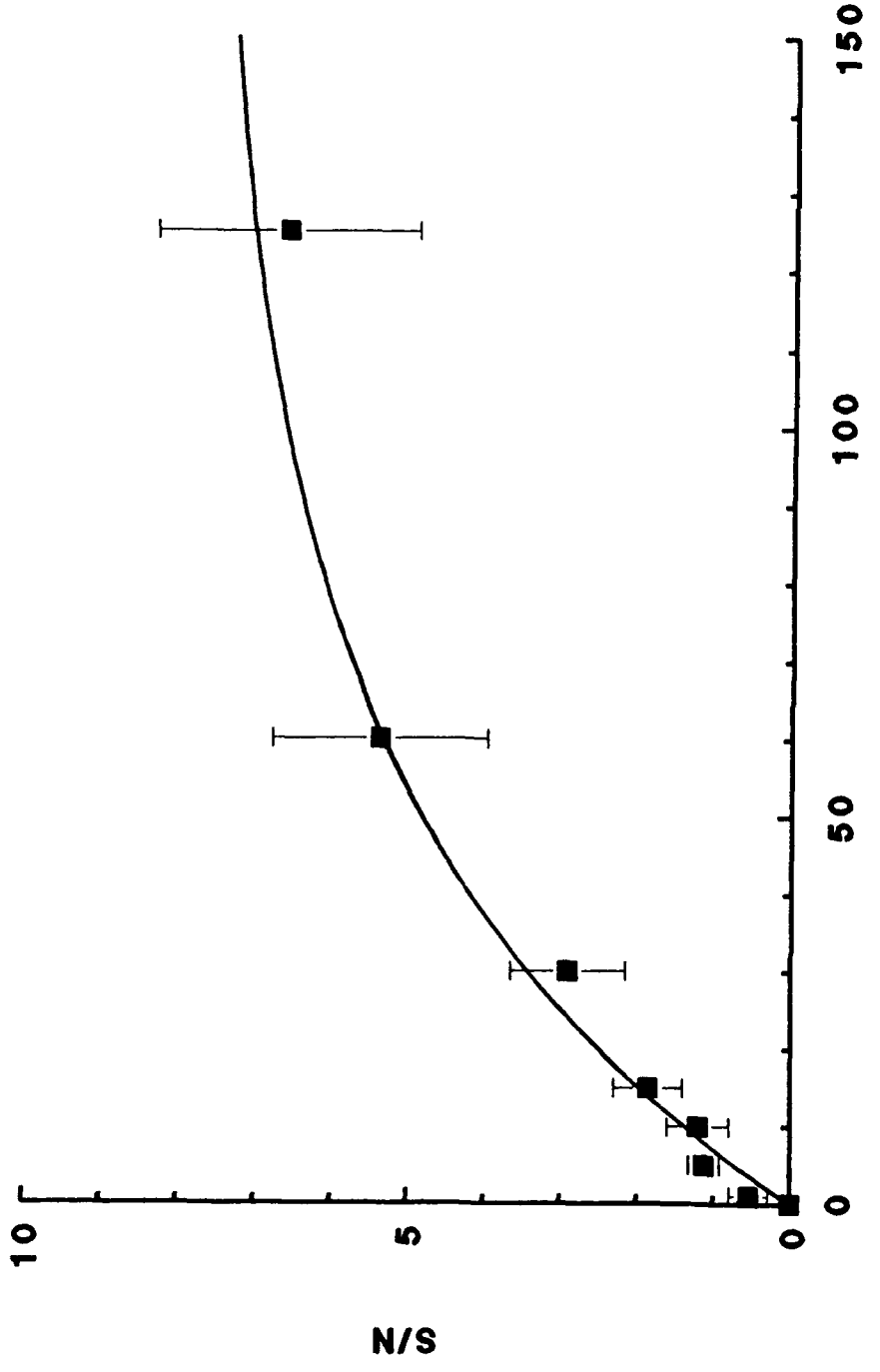

Figure 2

THE SIGNAL-TO-NOISE RATIO (S/N) VS INCUBATION TIME FOR THE LAP SENSOR ASSAY OF NDV. THE NOISE COMPONENT OF THE RATIO (N) WAS THE BACKGROUND (I.E., NO ANTIGEN) THE SIGNAL COMPONENT (S) WAS THE OUTPUT FOR A 50 ng SAMPLE OF NDV LESS THE NOISE. EACH POINT REPRESENTS THE QUOTIENT OF THE MEAN SIGNAL (n = 4) AND THE MEAN NOISE (n = 4). THE ERROR BARS REPRESENT ± SUM OF 1 SD OF THE SIGNAL AND 1 SD OF THE NOISE.

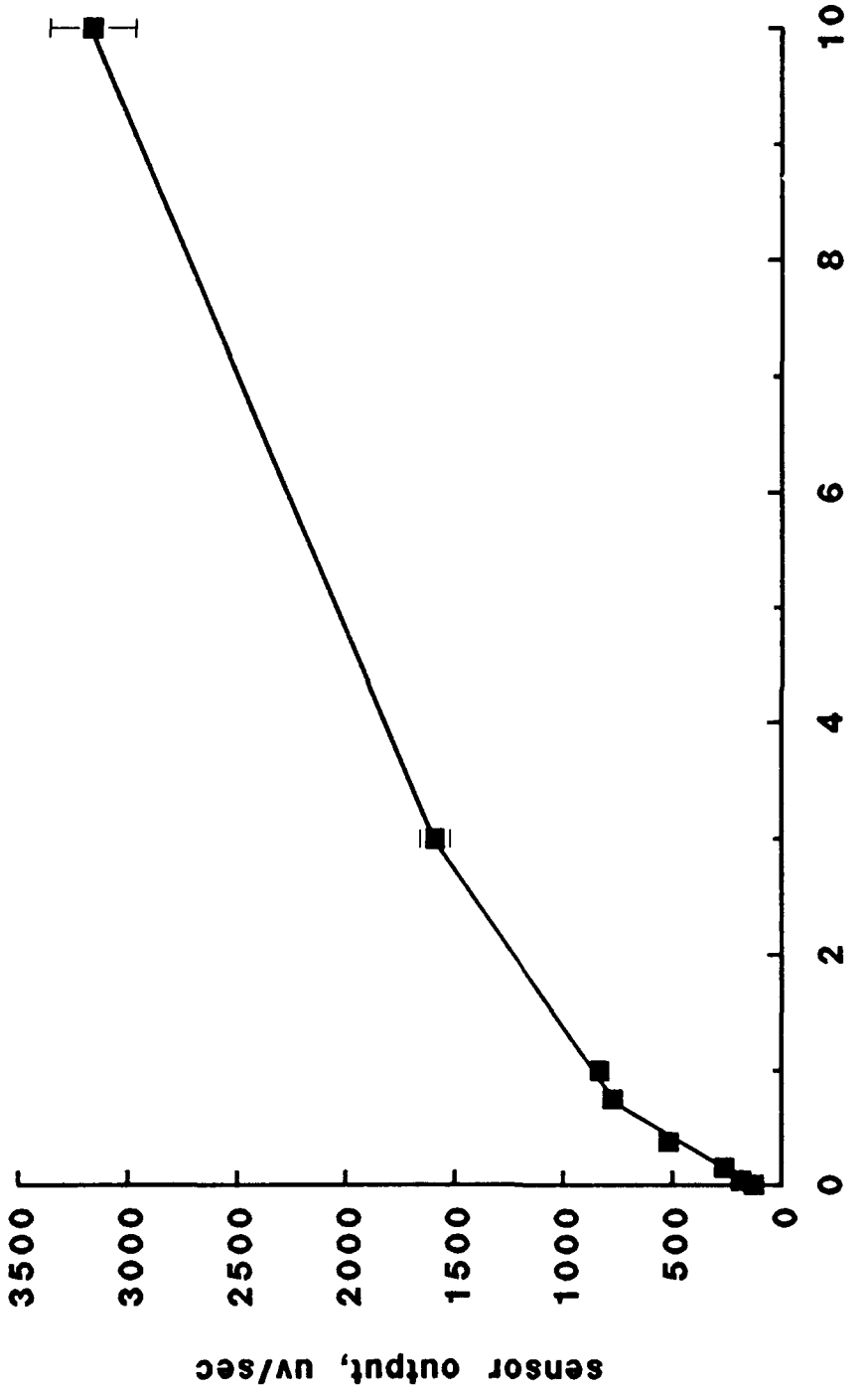

Figure 3

THE RESPONSE OF THE LAP SENSOR TO NDV. EACH POINT REPRESENTS THE MEAN OF THREE DETERMINATIONS. THE ERROR BARS, WHERE NOT MASKED BY THE DATA-POINT MARKERS, REPRESENT ± 1SD.

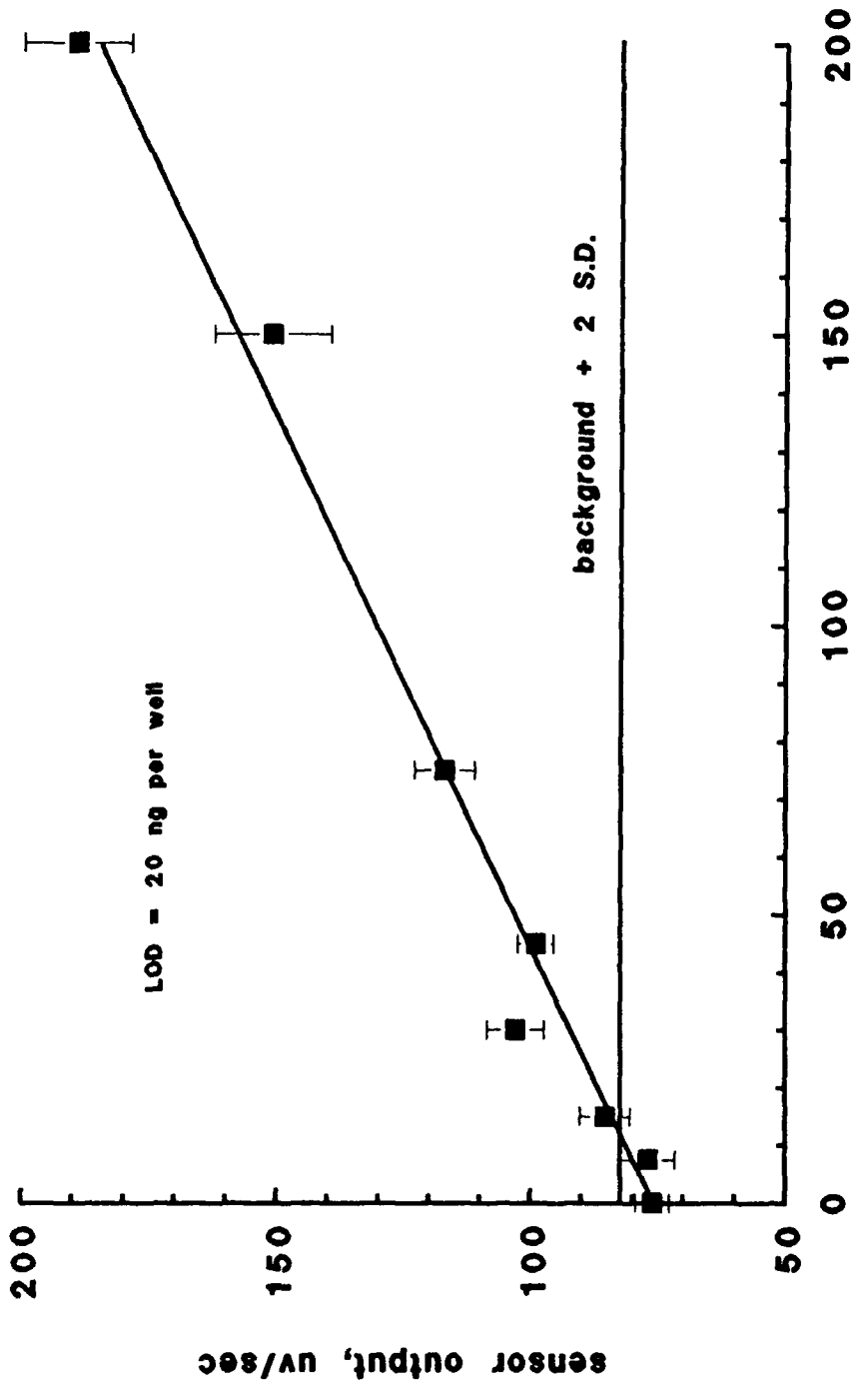

Figure 4

ASSAY OF NDV EMPLOYING A 1 MINUTE INCUBATION. EACH POINT REPRESENTS THE MEAN OF THREE DETERMINATIONS AND THE ERROR BARS REPRESENT ± 1SD. THE LINE DEFINING THE CALIBRATION CURVE IS GIVEN BY 76.3 + 0.54x, r^2 (CORRELATION COEFFICIENT) = 0.989. THE LOD (20 ng) WAS TAKEN TO BE THE INTERSECTION OF THE CALIBRATION CURVE WITH THE BACKGROUND PLUS 2 SD.

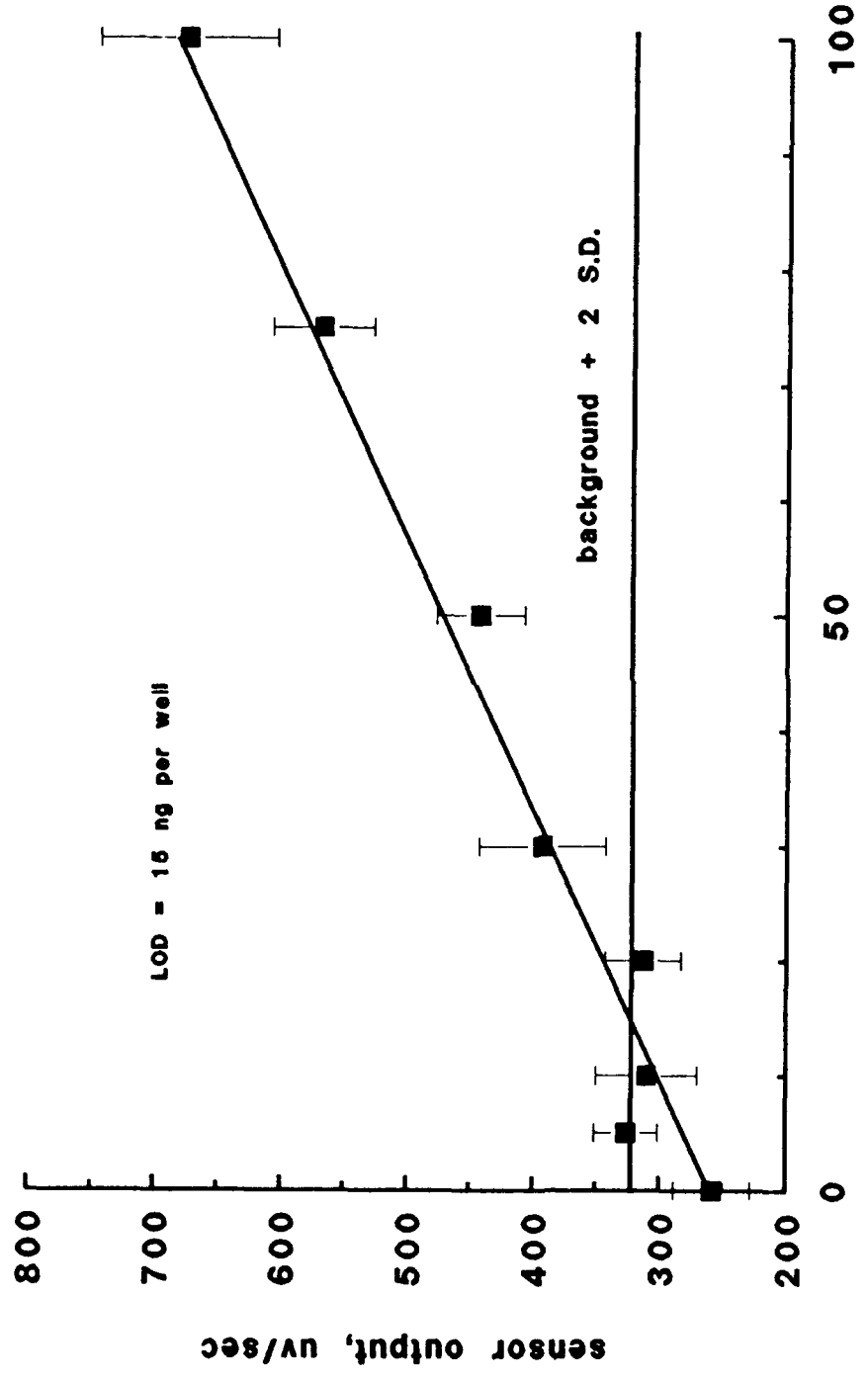

Figure 5

ASSAY OF NDV EMPLOYING A 5 MINUTE INCUBATION. EACH POINT REPRESENTS THE MEAN OF THREE DETERMINATIONS AND THE ERROR BARS REPRESENT ±1 SD. THE LINE DEFINING THE CALIBRATION CURVE IS GIVEN BY 262 + 4.2x, r^2 = 0.987. THE LOD (15 ng) WAS TAKEN TO BE THE INTERSECTION OF THE CALIBRATION CURVE WITH THE BACKGROUND PLUS 2 SD.

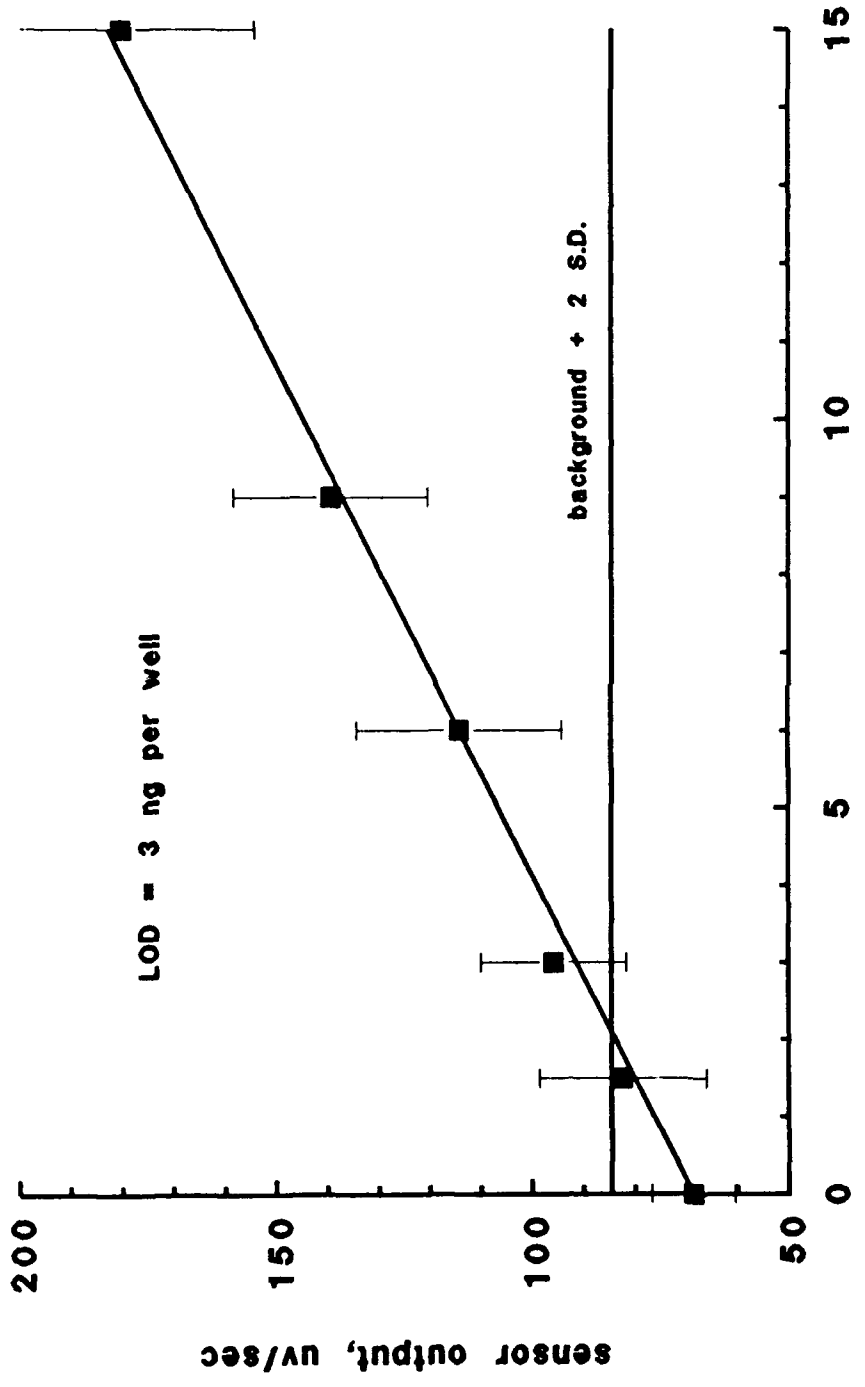

Figure 6

ASSAY OF NDV EMPLOYING A 60 MINUTE INCUBATION. EACH POINT REPRESENTS THE MEAN OF THREE DETERMINATIONS AND THE ERROR BARS REPRESENT ± 1 SD. THE LINE DEFINING THE CALIBRATION CURVE IS GIVEN BY 68.8 + 7.52x, r^2 = 0.998. THE LOD (3 ng) WAS TAKEN TO BE THE INTERSECTION OF THE CALIBRATION CURVE WITH THE BACKGROUND PLUS 2 SD.

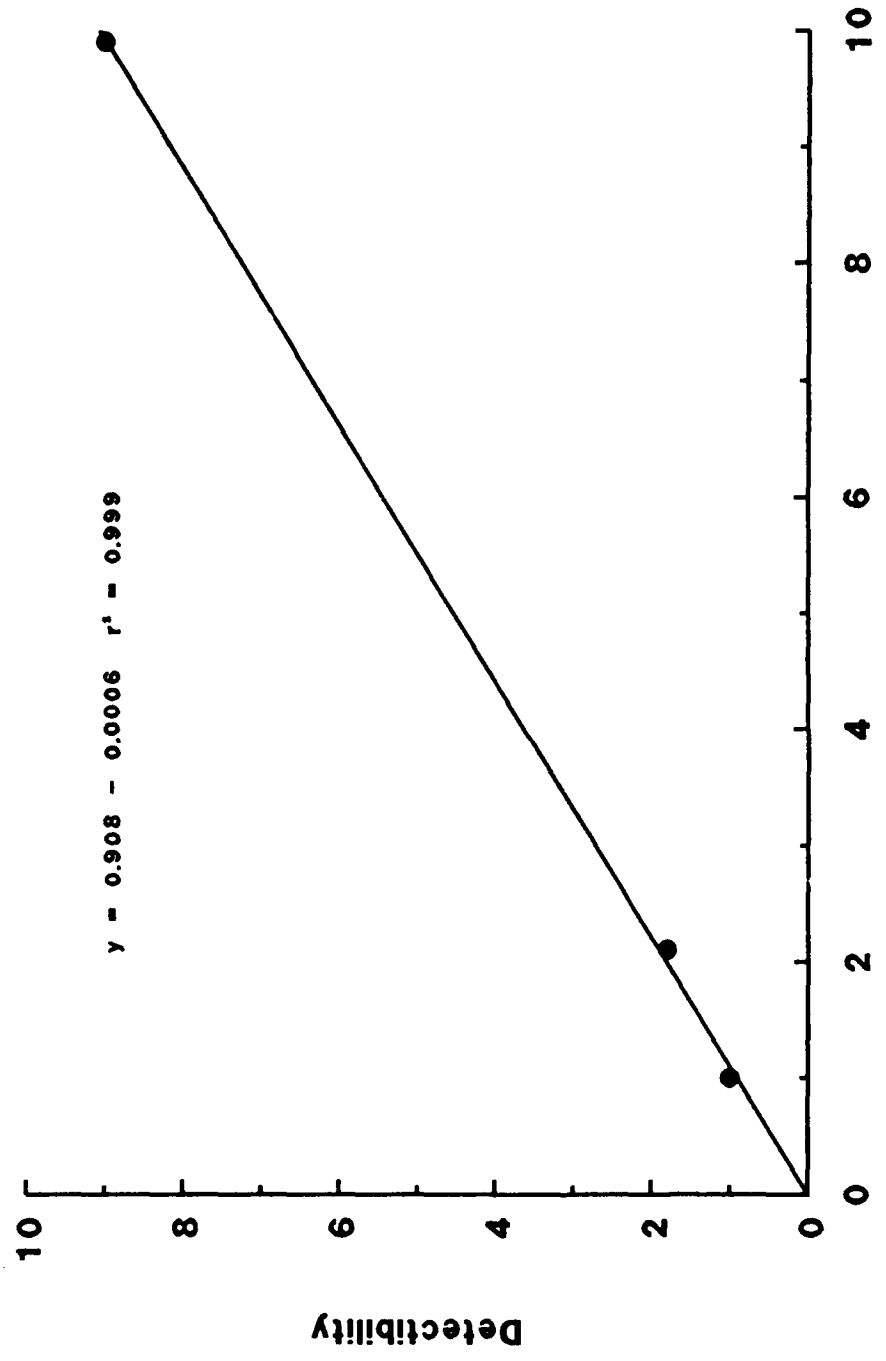

Figure 7

A PLOT OF DETECTABILITY VERSUS S/N FOR LAP SENSOR RESPONSE TO NDV. THE VALUES OF DETECTABILITY (RECIPROCAL OF LOD, SEE TEXT) AND S/N (FROM FIGURE 1) ARE RELATIVE TO THOSE OF THE 1 MINUTE ASSAY.

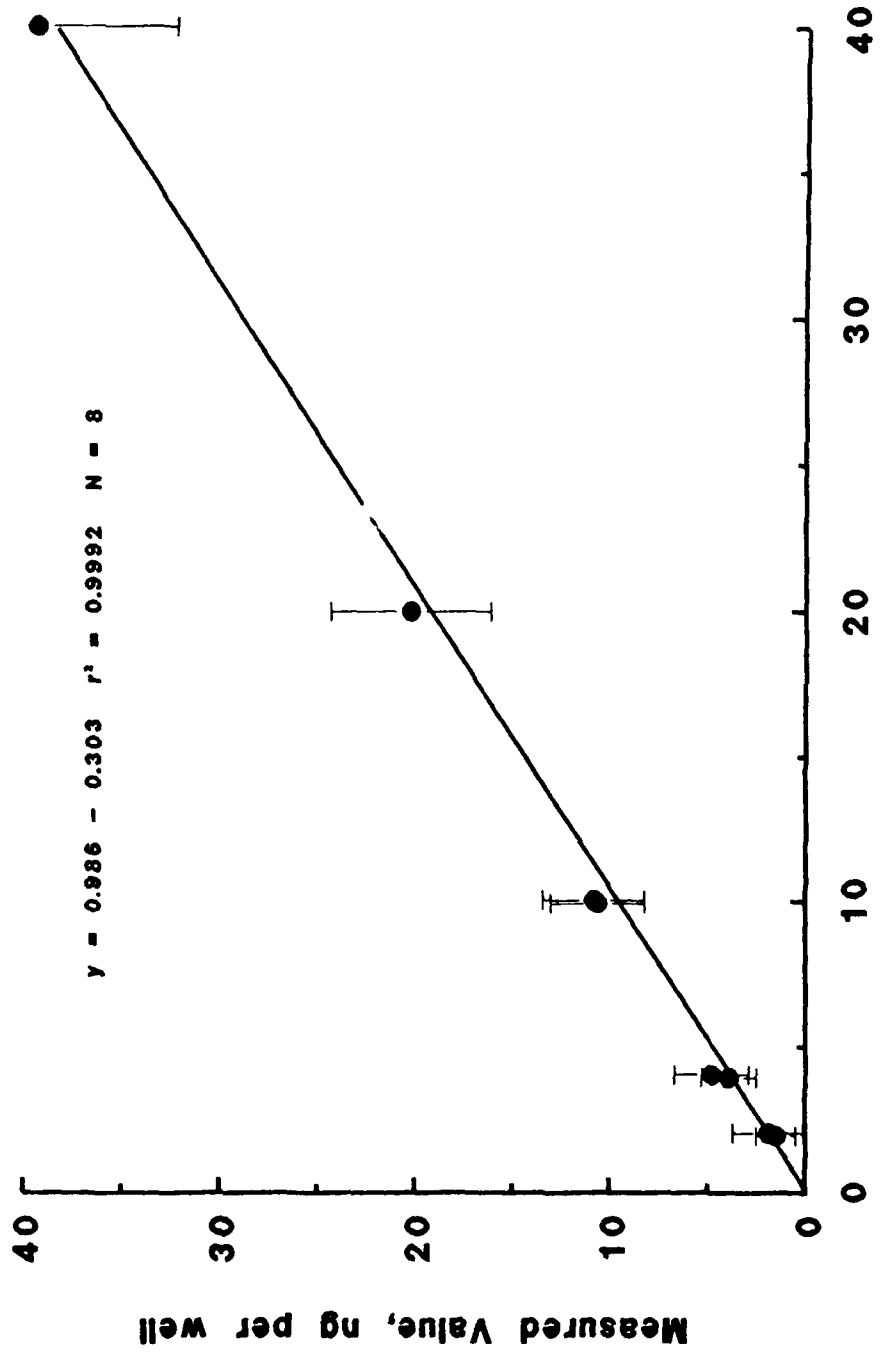

Figure 8

QUANTITATION OF NDV ON THE LAP SENSOR. EACH POINT REPRESENTS THE MEAN (n = 4) OF AN INDEPENDENT DETERMINATION OF NDV CONCENTRATION. THE ERROR BARS REPRESENT ± 1SD.

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A sandwich immunoassay for Newcastle Disease Virus (NDV) was developed using streptavidin-biotin mediated filtration capture and silicon-based semiconductor detection. The assays were carried out in one step whereby the immunoreagents and the antigen (NDV) were mixed together and incubated. The pH sensing capability of the detector, a light addressable potentiometric (LAP) sensor, was employed to detect the presence of immobilized urease-conjugated antibodies. Lower limits of detection (LOD) of the assay were determined as a function of incubation time. The LOD were approximately 27, 15 and 3 ng per well for 1, 5 and 60 minute incubations, respectively. The techniques described here have the potential to be adapted to an automated LAP sensor for use in the BioChemical Detector program.

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Immunoassay

ELISA

Newcastle Disease Virus

Biotin

Streptavidin